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(72) Inventors: EGGERS, Mitchell, D.; 10 Plum Cover Court, The Woodlands, TX 77381 (US). HOGAN, Michael, E.; 103 Golden Shadow Circle, The Woodlands, TX 77381 (US). EHRLICH, Daniel, J.; 11 Grant Place, Lexington, MA 02173 (US). HOLLIS, Mark, A.; 77 Lexington Road, Concord, MA 01742 (US). KOSICKI, Bernard, B.; 39 Fort Pond Road, Acton, MA 01720 (US). REICH, Robert; 146 Wellman Avenue, North Chelmsford, MA 01863 (US). BURKE, Barry, E.; 17 Sherburne Road, Lexington, MA 02173 (US).

(54) Title: METHODS AND APPARATUS FOR DETECTING AND IMAGING PARTICLES
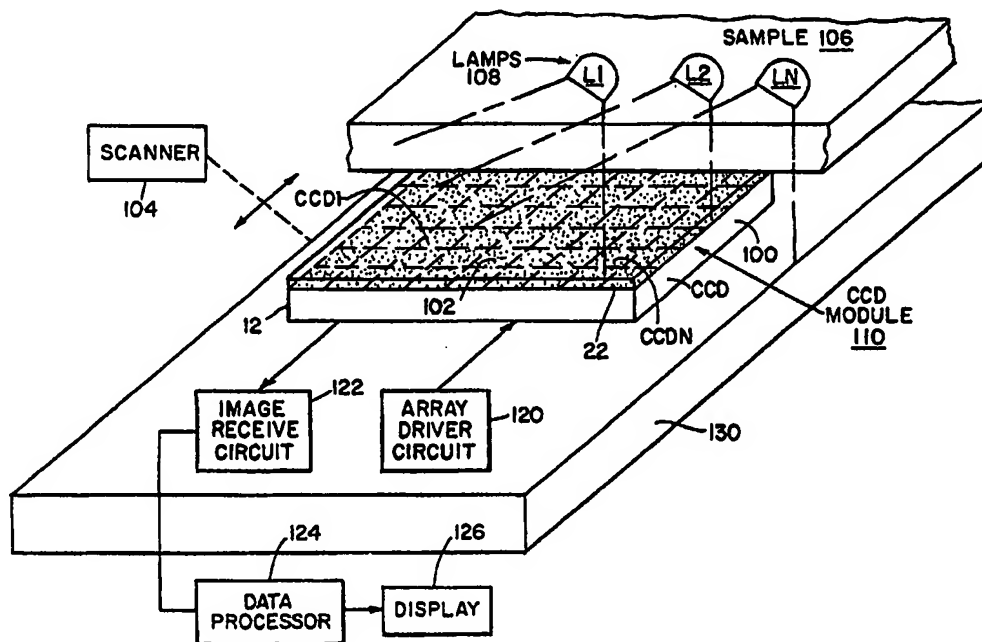
(57) Abstract

A method and apparatus for ultrasensitive detection and quantification of particle emissions from an emitter to produce a large format high resolution digital image of the spatial distribution of detected emissions is described. In one embodiment, the detector apparatus consist of a plurality of solid state imaging devices, such as an array of charge-coupled devices arranged in close proximity to the emitter in a manner to produce the relatively large format digital image without the need for a lens between the emitter and detector.

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METHODS AND APPARATUS FOR DETECTING AND IMAGING PARTICLES

Background of the Invention

The present invention is directly applicable to both
5   healthcare and scientific fields, such as, molecular
imaging. Regarding healthcare, previous attempts at
replacing conventional x-ray photography with digital
imaging systems such as computer tomography (CT) and
magnetic resonance imaging (MRI) have been somewhat
10 limited because of the extremely high detection
sensitivity required. Even with the advent of CT and MRI
digital imaging techniques, it has been estimated that
over 90% of the image diagnostic cases in healthcare
continue to be provided by conventional x-ray photography.
15 A need exists therefore for enhanced sensitivity in
medical radiography since the patient must be imaged in a
very short time in order to prevent blurring caused by the
motion of internal organs (typically less than 1/100
sec.), as well as reducing the risks associated with
20 radiation exposure.

An increase in sensitivity also offers many
advantages which can be exploited in scientific fields
such as x-ray crystallography, electron microscopy and the
imaging of labeled molecules. With increased sensitivity
25 complicated molecular structures can be analyzed by x-ray
diffraction imaging much faster than conventional
scintillation counting. Moreover, with increased
sensitivity, the radiation source intensity required is
significantly lower which allows transmission electron
30 microscopy without damaging the sample with electron rays.
That is, organic crystals and biopolymers are not broken
down during analysis with an electron microscope, thereby
allowing the use of the full theoretical resolution of
electron microscopes.

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Regarding molecular imaging, it is often desirable to rapidly detect and quantify one or more molecular structures in a sample. The molecular structures typically comprise ligands, such as cells, antibodies and
5   anti-antibodies. Ligands are molecules which are recognized by a particular receptor. Ligands may include, without limitation, agonists and antagonists for cell membrane receptors, toxins, venoms, oligo-saccharides, proteins, bacteria, and monoclonal antibodies. For
10 example, a DNA or RNA sequence analysis is very useful in genetic and disease diagnosis, toxicology testing, genetic research, agriculture and pharmaceutical development. Likewise, cell and antibody detection is important in disease diagnosis.

15 A.   Gel Electrophoresis
Analysis of many such molecular structures is often accomplished via gel electrophoresis; whereby biomolecules such as proteins and nucleic acids are separated by 1) disposing a sample, having an inherent charge polarity,
20 into a suitable gel, 2) applying an electrical potential across an electrode pair bounding the gel and 3) allowing the charged molecular components of the sample to migrate through the gel under the influence of the applied electric field. Separation is achieved simply from the
25 differential migration velocities exhibited by the molecular components. Once separated, the molecular components within the sample can be identified by detecting and quantifying the distribution of charged particles emitted from the labeled molecular components.

30 B.   Autoradiography
Several imaging procedures have been developed for detecting and quantifying the spatial particle emission distributions of such molecular samples. The most

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conventional imaging technique is autoradiography, whereby the molecular constituents are labeled with radioisotope tags such as ^{32}P and ^{35}S, and subsequently exposed to photographic film. Autoradiography provides a moderate

5   resolution image over a relatively large format area, but is plagued by several disadvantages. Due to the relative insensitivity of photographic film to beta particles, the time required for exposure can vary from hours to days. The exposure can be accelerated with the use of

10   intensifying screens, yet spatial resolution is significantly degraded. Typical spatial resolution for autoradiography is on the order of 1 to 10 millimeters. In addition, the resulting photographic image has to be read by a digital scanner for automation purposes.

15   C.   Gas Phase Ionization Beta Detection
          Gas phase ionization beta detection is another procedure for imaging radiolabeled molecules as disclosed in the following patent;
          [1]   Bolon, Process and Apparatus for Measuring
20   Surface Distributions of Charged Particle
                  Emitting Radionuclides, United States Patent
                  Number: 4,670,656, June 2, 1987, (incorporated
                  by reference herein).
          In this technique, beta particles are detected in two
25   separate phases, and the source of beta emission is then calculated by back propagation from the detector plates to the beta source. Since the beta particles are emitted at all angles from the surface horizontal, the back propagation results in a blurring effect that limits the

30   resolution, and is a function of the distance between the detector and the sample. Here the spatial resolution is on the order of 0.1 to 1 millimeters (a factor of 10 better than film) and provides a quantitative digital

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image. The sensitivity is approximately 10 to 100 times
better than film, yielding quantitative digital images at
least 10 times faster than film (minutes to hours).

D.   Storage Phosphor Screen Technology

5   Another method of quantitative detection and imaging
of radiolabeled molecules is the storage phosphor screen
technology, or phosphorimagers. Here a screen of phosphor
is placed in a metastable state when exposed to a
radioactively labeled sample. Following exposure, the
10   screen is read by exciting the phosphor by a laser and
imaging the photons emitted as the electrons return to the
ground state. Both strong ^{32}P and weak (^{14}C and ^{35}S) beta
particles can be imaged. The spatial resolution and
sensitivity is comparable to the gas phase beta detectors,
15   and offers many advantages. First, the variable limiting
the number of isotopic scans is the number of imager
plates. In essence many samples can be placed on a number
of imaging plates. Since the readout process is very
short (approximately 5 minutes), compared with the time
20   required for exposure of the storage phosphor matrix to
beta particle emission (tens of minutes to hours), the
machine is utilized more efficiently. Second, quantitation
of beta particles of lower energy are more amenable to
phosphorimagers. Tritium (^3H) can be imaged on a
25   phosphorimager, however, in this case the imaging plate is
not reusable. Thus, it is very expensive to detect ^3H on
a phosphorimager. Another disadvantage is optical self
attenuation, being a function of the screen thickness.

E.   Fluorescent Detection Systems

30   To circumvent the disadvantages of the above
detection and imaging methods based on radiolabeling,
several fluorescent detection systems have been developed.

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Here the molecular components are labeled with fluorescent
dyes with distinct emission bands. For example, in DNA
sequencing, 4 fluorophores are used to label each of 4
dideoxy sequencing reactions. Following gel
5   electrophoresis, the sample is radiated with a laser and
images are produced from signals obtained with a
photomultiplier tube (PMT). The wavelength selectivity,
corresponding to distinct molecular constituents, is
obtained by positioning bandpass filters in front of the
10 PMT. Further automation can be achieved by multiplexing
several samples per run, as well as automating the base
calling with computer algorithms, characteristic of the
Applied Biosystems, Inc. DNA sequencer.

More recently, several fluorescent detection and
15 imaging techniques have been disclosed utilizing charge-
coupled-device (CCD) cameras in conjunction with gel
electrophoresis as described in the following listed
patents (incorporated by reference herein):

[2]   Mackay, *Analysis of Samples by Electrophoresis*
20   *Using a Charge Coupled Device,* United States
Patent Number: 4,874,492, October 17, 1989.

[3]   Yamamoto, *et al., Electrophoresis Pattern*
Analyzer for Genetic Material, United States
Patent Number: 5,061,067, October 29, 1991.

25 [4]   Pentoney, Jr. *et al., Detection of Radioisotope*
Labeled Components in a Capillary, United States
Patent Number: 5,143,850, September 1, 1992.

In each case a cooled CCD camera is used to image the
fluorescently tagged molecular constituents in the gel.
30 Advantages of a CCD-based detection and quantitative
imaging approach include: 1) avoidance of mutagenic
radioactive labels, 2) high sensitivity (comparable to
PMT), 3) fast exposure time (seconds), 4) linear response
over wide dynamic range (5 orders of magnitude), 5) low

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noise, 6) high quantum efficiency, and 7) fast data
acquisition (megapixels/sec).

Several articles have recently appeared describing
the employment of CCD cameras for electrophoresis
5   applications including the following,(incorporated by
reference herein):

[5]   Jackson, P., Urwin, V.E., Mackey, C.D., Rapid
       Imaging Using a Cooled Charge-Coupled Device of
       Fluorescent Two-dimensional Polyacrylamide Gels
10   Produced by Labeling Proteins in the First
       Dimensional Isoelectric Focusing Gels with the
       Flurophore 2-Methoxy-2, 4-Diphenyl-3(2H)
       Furanone, *Electrophoresis* 9(7), 330-339, 1988.

[6]   Chan, K.C., Koutny, L.B., and Yeung, E.S., On-
15   Line Detection of DNA in Gel Electrophoresis by
       Ultraviolet Absorption Utilizing a Charge-
       Coupled Device Imaging System, *Anal. Chem.*, 63,
       746-750, 1991.

where the gel is radiated with a UV laser and the
20   absorption of the DNA constituents is recorded digitally
with a CCD camera providing on-line detection and imaging,

[7]   McGregor, D.A. and Yeung, E.S., Interactive
       Control of Pulsed Field Gel Electrophoresis via
       Real Time Monitoring, *Anal. Chem.*, 64, 1-6,
25   1992.

where a CCD camera has been used for pulsed field gel
electrophoresis wherein the electric field is alternated
interactively causing the DNA fragments to snake through
the gel at approximately 10 times faster than conventional
30   electrophoresis,

[8]   Cheng, Y.F., Piccard, R.D. and Vo-Dinh, T.,
       Appl. Spectrose 44:755-765, 1990.

[9]   Sweedler, J.V., Shear, J.B., Fishman, H.A.,
       Zare, R.N. and Scheller, R.H., Fluorescence
35   Detection in Capillary Zone Electrophoresis

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Using a Charge-Coupled Device With Time-delayed
Integration, *Anal. Chem.*, 63, 496-502, 1991.

[10] Kostichka, A.J., Marchbanks, M.L., Brumley, Jr.,
R.L., Drossman, H. and Smith, L.M., High Speed
Automated DNA Sequencing in Ultrathin Slab Gels,
Biotechnology, 10:78-81, 1992.

[11] Karger, A.E., Harris, J.M. and Gesteland, R.F.,
Multiwavelength Fluorescence Detection for DNA
Sequencing Using Capillary Electrophoresis, *Nuc.
Acids Res.*, 19:4955-4962, 1991.

where the presence of a CCD camera has appeared in
capillary gel electrophoresis applications wherein very
thin fused silicon capillaries are filled with denatured
polyacrylamide gel which facilitate substantially larger
electric fields due to the efficient heat transfer
properties of the small capillaries. Consequently,
throughput is increased by an order of magnitude due to
the combination of faster molecular mobilities in the gel
(26 times faster migration), multiple wavelength
fluorophores, and on-line parallel CCD camera imaging.
Also, no moving parts are required, such as a filter
wheel, since a wedge prism assembly is used to provide
distinct emission bands for CCD camera imaging.
Alternatively, the multiple wavelength emissions can be
discriminated by calculating the condition number to avoid
the use of bandpass filters.

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Collectively, these CCD camera-based approaches for detection and quantitative imaging of particle emissions from molecular samples circumvent many of the limitations of autoradiography, gas phase ionization beta detection,
5   phosphorimaging and PMT fluorescence detection. However, limitations do persist including the low collection efficiency inherent to any CCD technique having long focal lengths (approximately 75 cm) from the sample (emitter) to the detector, cryogenics required to suppress CCD dark
10   currents, and the expense incurred for the lens optics required with long focal lengths.

F.   CCD Lens-Based Document Imaging
      CCD technology utilizing optical lenses for imaging documents has been well documented and applied in
15   conventional document scanners, copying machines, and facsimile machines, as disclosed in the following patents (incorporated herein by reference):
            [12] Suzuki, *Image Reading Device,* United States
                  Patent Number: 4,772,958, September 20, 1998.
20   [13] Suzuki, *Image Reading Apparatus,* United States
                  Patent Number: 4,675,745, June 23, 1987
            [14] Hirota, *Image Reading Apparatus Having Plural*
                  Line Image Sensor Chips, United States Patent
                  Number: 5,003,380, March 26, 1991.
25   [15] Hornbaker, III et al., *Scanning Apparatus Using*
                  Multiple CCD Arrays and Related Method, United
                  States Patent Number: 5,144,448, September 1,
                  1992.
            [16] Suzuki *et al., Image Reader Using a Plurality of*
30   *CCDs,* United States Patent Number: 4,849,820,
                  July 18, 1989.
            [17] Suzuki *et al., Image Reader Using a Plurality of*
                  CCDs, United States Patent Number: 4,774,592,
                  September 27, 1988.

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Although these lens-based CCD approaches often employ
large format imaging sensors, they are not applicable to
imaging charged particles emitted from radioisotope,
chemiluminescent or fluorescent labeled molecular samples.

5   Summary of the Invention
The present invention provides an ultrasensitive
detection, quantitative imaging and spectroscopy method
and apparatus that yields high resolution digital images
of the spatial distribution of particle emissions from
10   relatively large format samples in a minimal amount of
time and expense.
The apparatus is comprised of a lens-less imaging
array comprising a plurality of solid state imaging
devices, such as an array of CCDs, charge injection
15   devices (CIDs), photodiode arrays, amorphous silicon
sensors or the like. The array is disposed in close
proximity to the sample and is comparable in size to the
area of the sample to be imaged. In this manner a
relatively large format digital image of the spatial
20   distribution of sample emission or absorption is produced
without requiring the use of one or more lenses between
the sample and the imaging array. The apparatus offers 1)
high sensitivity (10^3 to 10^4 times more than photographic
film, 10 - 100 times more than gas phase ionization
25   detection and phosphorimagers), 2) high throughput
(seconds instead of minutes to hours for gas phase
ionization detectors and phosphorimagers, and hours to
days for photographic film), 3) linear response over a
wide dynamic range (4 to 5 orders of magnitude), 4) low
30   noise, 5) high quantum efficiency and 6) fast data
acquisition. Moreover by placing the imaging array in
proximity to the sample, the collection efficiency is
improved by a factor of at least 10 over any lens-based

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technique such as found in conventional CCD cameras. That is, the sample (emitter or absorber) is in near contact with the detector (imaging array), eliminating conventional imaging optics such as lenses and mirrors.

5   The inventive apparatus can be used for detecting and quantitatively imaging radioisotope, fluorescent, and chemiluminescent labeled molecules, since a lens-less CCD array apparatus is highly sensitive to both photons and x-ray particles. Hence a single imaging instrument can be

10   used in conjunction with numerous molecular labeling techniques, ranging from radioisotopes to fluorescent dyes.

Two categories of embodiments of the invention are disclosed. The first embodiment entails a *static* platform

15   whereby a plurality of imaging devices are arranged in a relatively large format area comparable to the sample size.

The second category of embodiments of the invention entails a *dynamic* platform that enables a smaller set of

20   imaging devices to image a relatively large format sample by moving either the array of imaging devices or sample, relative to one another.

In summary, the dynamic embodiment of the invention generally concerns a method and apparatus for

25   ultrasensitive detection, high resolution quantitative digital imaging and spectroscopy of the spatial and/or temporal distribution of particle emissions or absorption from/by a sample in a relatively large format. The apparatus of the invention includes:

30   a)   a large area detector array for producing a relatively large image of detected particle distribution without the use of optical lenses;

           b)   a scanner for moving either the sensor array or the sample in a manner for efficient imaging;

35   and

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c)   a source of energy for exciting the sample or
      providing absorption by the sample.

Optimally the ratio of detector array size to sample
image is 1 for a static format and less than 1 for a
5   dynamic format.

Brief Description of the Drawings

FIG. 1A is a schematic sectional view of a portion of
a static embodiment of the invention.

FIG. 1B is a plan view of a static embodiment,
10   showing 4 columns of CCD chips.

FIG. 1C is a plan view of a static embodiment with 4
columns and 5 rows of CCD chips.

FIG. 2A is a schematic diagram illustrating the
geometry of a lens coupled detector system of the prior
15   art.

FIG. 2B is a schematic diagram illustrating the
geometry of a lens-less detector system of the present
invention.

FIG. 3 is a schematic diagram of a dynamic embodiment
20   of the invention.

FIG. 4 is a schematic of an alternate embodiment of
the invention.

FIG. 5 is a sectional view of a conventional wire
bond technique applied to the present device.
25   FIG. 6 is a sectional view of a modification of FIG.
5 to accommodate wire bonding of the present detector
system.

FIG. 7 is a sectional view of a low profile wire bond
of the present invention.
30   FIG. 8 is a sectional view of a detector/sample using
the bonding system of FIG. 7.

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FIG. 9 is a sectional view of a detector/sample with a low profile bond and isolated imaging on a dynamic platform.

FIG. 10 is a sectional view of a detector/sample for
5   a backside illuminated approach.

Description of the Invention

Sensor System

A preferred embodiment of a sensor system 10 of the invention consists of a plurality of CCD arrays
10   CCD1...CCDN assembled in a large format module as illustrated in FIGS. 1A-1C. Individual CCD arrays are closely aligned and interconnected in particular geometries to form a relatively large (greater than $1cm^2$) format imaging sensors of the linear array type 10B or the
15   two dimensional row and column type 10C shown in FIGS. 1B and 1C respectively.

Each CCD array CCD1...CCDN is formed, in the conventional manner, by growing and patterning various oxide layers 14 on a Si wafer/substrate 12. CCD gate
20   electrodes 10 are then formed by deposition of polysilicon or other transparent gate material on the gate insulator or field oxide 14. A dielectric or polymer layer 18, preferably of light transmissive material such as silicon nitride or glass, SiO_2 or polyamide is then formed over
25   the electrodes 16.

Preferably, in a labeled molecule embodiment a filter shown in dotted lines 17, which may be formed of an aluminum or tungsten metal grating, or dielectric multilayer interference filter, or absorption filter, is
30   formed in the dielectric layer 18 between the surface and the metal electrode 16. The filter is adapted to block the excitation radiation and pass the secondary emission from the sample 20. In a static platform embodiment, the

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sensor module remains fixed with respect to the sample.
Hence to achieve the relatively large imaging format, a
plurality of imaging devices CCD1...CCDN must be arranged
in a module as illustrated in FIGS. 1B and 1C. The module
5   can be packaged for easy installation to facilitate
multiple modules, each for specific applications. Various
tiling strategies have been documented and can be employed
to minimize the discontinuity between devices, such as
described in:

10   [18] Burke, *et al.*, "An Abuttable CCD Imager for
          Visible and X-Ray Focal Plane Arrays," IEEE
          Trans. on Electron Devices, *38*(5):1069 (May
          1991)

          As illustrated in FIG. 1A, a sample 20 is placed in
15   proximity to the CCD array sensor 10. For example, the
sample may be the chest of a patient for clinical
radiology applications, or a gel having labeled molecular
constituents for analysis. The sample can be excited by
an external energy source or can be internally labeled
20   with radioisotopes emitting energetic particles or
radiation, or photons may be emitted by the sample when
labeled with fluorescent and chemiluminescent substances.
Conversely, direct absorption may be used to determine
their presence. In this case, the absence of illuminating
25   radiation on the detector may constitute the presence of a
particular molecule structure. Preferably the sample can
be physically separated from the CCD detector by (an
optional) thin isolation plate 22, such as glass or
quartz, which is transparent to the particle emission.
30   The CCD detection and imaging arrays CCD1-CCDN
generate electron-hole pairs in the silicon 12 when the
charged particles or radiation of energy hυ shown by the
"asterisk" 32 arising from or transmitted by the sample
are incident (arrows 30) on the CCD gates 16.
35   Alternatively, the CCDs can be constructed in a back

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illuminator format whereby the charged particles are
incident in the bulk silicon 12 for increased sensitivity.
The liberated photoelectrons 34 are then collected beneath
adjacent CCD gates 16 and sequentially read out on a
5   display (not shown) by display module 36 in the well-known
manner.

        Silicon based CCD's are preferred as the solid state
detection and imaging sensor primarily due to the high
sensitivity of the devices over a wide wavelength range of
10   from 1 to 10,000Å wavelengths. That is, silicon is very
responsive to electromagnetic radiation from the visible
spectrum to soft x-rays. Specifically for silicon, only
1.1 eV of energy is required to generate an electron-hole
pair in the 3000 to 11000Å wavelength range. Thus for
15   visible light, a single photon incident on the CCD gate 16
will result in a single electron charge packet beneath the
gate, whereas for soft x-rays, a single beta particle
(typically KeV to MeV range) will generate thousands to
tens of thousands of electrons. Hence the silicon CCD
20   device provides ultrasensitive detection and imaging for
low energy alpha or beta emitting isotopes (^{3}H, ^{14}C, ^{35}S) as
well as high energy alpha or beta emitting isotopes (^{32}P,
^{125}I). Consequently, the CCD is both a visible imager
(applicable to fluorescent and chemiluminescent labeled
25   molecular samples) and a particle spectrometer (applicable
to radioisotope labeled samples as well as external x-ray
radiated samples). In fact the CCD is the only sensor
that can provide simultaneous imaging and spectroscopy in
the same image.
30   In addition to the high sensitivity, the CCDs offer a
wide dynamic range (5 orders of magnitude) since the
charge packet collected beneath each pixel or gate 16 can
range from a few to a million electrons. Furthermore, the
detection response is linear over the wide dynamic range

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which facilitates the spectroscopy function, since the
amount of charge collected is directly proportional to the
incident photon energy. Hence, no reciprocity breakdown
occurs in CCDs, a well-known limitation in photographic
5   film.

     SNR Calculations

     Several signal-to-noise ratio (SNR) expressions are
presented below to convey the advantages of the preferred
lens-less CCD embodiment for ultrasensitive detection and
10 imaging of samples in a relatively large format.
     ·X-ray Excitation
     The first approach requires either external x-ray
sample excitation or the attachment of a radioactive label
to the sample constituents. Here the pixels (gates) 16 on
15 the CCD arrays accumulate electrical charge proportional
to the radiation products absorbed from the sample.
Hence, charge particles are detected almost
instantaneously by automatically addressing and reading
the charge.
20   The SNR for the lens-less CCD approach used with x-
ray excitation can be expressed once the integration time
is determined. Assume the sample is labeled with ^{32}P. Let
N_l = sample labeling density (10^{11} labeled molecules/cm^2); T
= ^{32}P decay time (18 days); Ap = pixel area (27 μm)^2; A_l =
25 area of active labeling per molecular component; A = total
area of single molecular component site (including guard
pixels); t = integration time (sec); I_d = dark current
(1nA/cm^2); q = electron charge (1.6x10^{-19} coul) and σ_r^2 =
read noise (10 electrons/pixel). The number of
30 radioactive events within integration time t occurring in
an active area A_l is given by

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$$N_e(t) = \frac{1}{2}N_t A_1 (1 - e^{-t/T})$$

and for $t \ll T$

$$N_e(t) \approx \frac{1}{2}N_t A_1 \frac{t}{T}.$$

To achieve high probability of detection at least 10 events are required, yielding the net integration time for an approximate $(100\mu m)^2$ label area $A_l = 16A_p$

$$t = \frac{2(10)T}{A_1 N_t} \approx 3 \text{sec}.$$

5   Caution must be exercised in designing the array to avoid crosstalk between pixels, since the beta particle creates a trail of photocharge that is collected across several pixels. The corresponding SNR calculated with the guard pixels to avoid crosstalk $(A = 20^2 A_p)$ can be

10   expressed

$$SNR = \frac{SNe(t)}{\sqrt{SNe(t) + \sigma^2_r + \frac{I_d t A}{q}}} \approx 122$$

where S = electrons generated per beta particle (single event). From experimental results, $S = 90,000$ electrons for a 1.7 MeV beta particle.

The dark current contribution to the noise can be
15   decreased and crosstalk suppressed by framing the CCD very quickly using a shorter integration time $\tau \ll t$. By fast framing, the CCD pixels become single event detectors

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since the number of collected photoelectrons in a pixel
remains larger than either the dark current or read noise
and the probability of one or more events is small in the
duration of τ seconds. Although the read noise increases,
5   the charge-to-voltage noise associated with a single pixel
readout can be less than 10 electrons. Also, the pixels
can be designed to minimize the variation in electrons
collected per beta particle event.

          Such technique is well suited to the x-ray excitation
10   mode since the beta particle events are relatively
infrequent and easily distinguished from noise frames.
Moreover, crosstalk is further suppressed by the technique
since the probability of beta events occurring
simultaneously in neighboring sample component sites
15   decreases with decreasing integrating time. The use of
lower energy beta particles (^{35}S) will also suppress
crosstalk. Therefore the density of the labeling can be
increased to accommodate thousands of distinct molecular
components on a single sample when employing single event
20   detection.

          Consequently, a ^{32}P labeled sample can be detected in
seconds time throughout thousands of sites on a large
format CCD imaging module operating at room temperature
with sufficient SNR. A similar analysis can be conducted
25   for a sample externally excited by a x-ray source by
substituting the radiated energy level incident on the
imaging module for the signal electron density S.

          •*Fluorescent Excitation*

          The second approach analyzed involves the use of
30   fluorescent-labeled receptors attached to the sample
components. The fluorescent labels can be attached
covalently or through intercalation.

          The detection procedure for using the fluorescent
labels begins by analyzing the optical absorption spectrum

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of the chosen label. The regions in the spectra of high
absorption are used to select the illumination source
wavelength. For many of the labels, an ultraviolet (UV)
light source provides maximum absorption and emission is
5   in the red spectrum (e.g., 630 nm). These spectral
regions of excitation and detection enhance SNR, since the
polysilicon gates of the CCD substrate naturally absorb UV
light and the line grating fabricated on the chip can
provide optical filtering near the 630 nm region.
10 Consequently, sensitivity can be improved by employing
fluorescent markers, choosing the optimal excitation
wavelength and the corresponding detection filter
implemented directly on the CCD chip. Finally, the charge
accumulated at the pixels is proportional to the number of
15 photons detected from the fluorescence of the molecular
constituents of the sample.

   The SNR for the lens-less CCD approach in the
fluorescent mode of operation is given by

$$SNR = \frac{P\sigma N_t \eta_d \eta_f \eta A_p t}{\sqrt{P\sigma N_t \eta_d \eta_f \eta A_p t + \dfrac{I_d A_p t}{q} + \sigma^2_r}}$$

where P = pump power (laser or bulb) (photons/cm^2·sec); σ =
20 absorption cross section of the dye (20×10^{-16} cm^2); η_d =
detection quantum efficiency (4 electrons/10 photons at
700 nm emission); η_f = fluorescence quantum yield (1); η =
CCD collection efficiency (50%); I_d = dark current noise
(1nA/cm^2 at room temperature; h = Planck's constant
25 (6.6×10^{-27} erg·sec).

   Thus, in order to obtain a SNR = 100 to ensure high
detection probability, the power density required of the
illumination source for a one second integration time is
given by:

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$$P=\frac{1}{2}\left[\frac{SNR^2}{\alpha A_p t}+\left[\left(\frac{SNR^2}{\alpha A_p t}\right)^2+\frac{4 I_d SNR^2}{q\alpha^2 A_p t}\right]^{\frac{1}{2}}\right]$$

$= 9.3 \times 10^{13}$ photons / $cm^2 \cdot S$

where

$\alpha = \sigma N_i \eta_d \eta_f \eta$.

Specifically, for a 308 nm UV source, the power density

5   requirement becomes

$$P_{UV}=P\frac{hc}{\lambda}=61 \mu W/cm^2$$

Consequently, the excitation required to ensure
sensitive detection can be met in theory using a 61 $\mu W/cm^2$
UV source. This can be provided by a filtered mercury
arc, or else by one of several relatively inexpensive UV

10   laser sources.
     ·*Chemiluminescent Excitation*
Chemiluminescence is the conversion of chemical energy
into electromagnetic radiation. In these types of
reactions, electrons are excited via a chemical reaction,

15   and light is emitted upon return of the excited electrons
to the ground state. Unlike other chemiluminescent
modalities, enzyme-catalyzed 1,2-dioxetane derivatives can
produce a light signal that can last from hours to days.
The wavelength of emitted light is near 477 nm, and the

20   emission can be controlled by controlling the pH.
     In addition to no radioactivity exposure, sensitivity
is greater for chemiluminescent methods as compared to
radioactive methods. Moreover, this method is relatively
simple to perform (reagents and equipment are

-20-

inexpensive). Finally this method has a low background
noise level and wide dynamic range.

The SNR for the lens-less CCD approach for a 0.1
second integration time in the chemiluminescent mode of
5   operation follows the expression obtained for the
fluorescent approach and is given by (assuming negligible
read noise)

$$SNR = \frac{P_e \sigma N_t \eta_d \eta_c \eta \, (A_p t)^{\frac{1}{2}}}{\sqrt{P_e \sigma N_t \eta_d \eta_c \eta + \frac{I_d}{q}}} = 838$$

where P_e = enzymatic reaction turnover rate (3000
reactions/enzyme·sec); σ = enzyme to molecule coupling
10   ratio (10 enzymes/molecular component); η_d = detection
quantum efficiency (13 electrons/100 photons @ 477 nm
emission); η_c = chemiluminescent quantum yield (5
photons/1000 reactions); η = CCD collection efficiency
(50%). The values for the enzymatic reaction turnover
15   rate and chemiluminescent quantum yield are typical for
the dioxetane reagent.

Consequently with the above constants, the
chemiluminescent approach appears feasible for detecting
thousands of molecular constituents within a sample in
20   proximity to the CCD imaging module operating at room
temperature.

Lens-less Operation

Improved detection sensitivity beyond traditional CCD
camera-based imaging approaches is attributable in the
25   present invention to the coupling of the detector (CCD
arrays) to the emitter (molecular sample 20). Hence by
avoiding traditional optics (lenses, mirrors), the

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proposed system offers a much improved collection
efficiency.

The collection efficiency η is measure of an
instrument's capability to capture electromagnetic
5   radiation and is primarily a function of geometry. The
geometrical configurations for lens-based instruments
(epifluorescent microscope, confocal microscope, CCD
camera and the like) and the lens-less CCD imaging module
are illustrated in FIGS. 2A and 2B, respectively.

10   Collection efficiency is typically expressed as a
ratio of solid angles

$$\eta = \frac{\Omega}{4\pi}$$

where

$$\Omega = \iint \frac{\cos\psi \, dS}{r^2}$$

and Ψ is the angle between the surface normal vector and
the position vector. For the lens configuration ($\Psi = 0$)

$$\Omega_{lens} = \int_0^{2\pi}\int_0^{\phi_0} \frac{r^2\sin\phi \, d\phi \, d\theta}{r^2}$$

$$= 2\pi(1-\cos\phi_0)$$

15   where ϕ_0 = half angle of the cone of rays entering the
lens. Consequently, the collection efficiency of the
lens-based systems is

-22-

$$\eta_{lens} = \frac{1}{2}(1 - \cos\phi_0).$$

In contrast, the direct proximity lens-less CCD approach yields the largest half angle ($\phi_0 = 90°$) resulting in the collection efficiency

$$\eta_{CCD} = \frac{2\pi}{4\pi} = \frac{1}{2}.$$

5   The difference between these configurations becomes quite apparent once numerical apertures are provided. For example, suppose a high quality lens with a numerical aperture NA = 0.3 is employed. Since

$$NA = n\sin\phi_0$$

then

$$\eta_{lens} = \frac{1}{2}\left[1 - \cos\left(\sin^{-1}\left(\frac{NA}{n}\right)\right)\right] = 2.3\%$$

which is substantially lower than 50% achieved by the
10   lens-less CCD approach. Furthermore, if the numerical aperture is increased to 0.4, typical of a $100K photolithography lens for producing 1M byte memory chips, the collection efficiency increases to only 4.2%. Consequently, the integrated CCD approach provides at
15   least a 10x collection advantage over any lens-based system, and allows the additional imaging of radioactively labeled samples.

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Instrumentation Platforms

One of two preferred embodiments for housing the
above described detection and imaging sensor is
illustrated schematically in FIG. 3. The embodiment of
5   FIG. 3 consists of:

    a)   a relatively large format imaging sensor 100
        formed of a CCD module 110 comprised of a
        plurality of smaller imaging devices
        CCD1...CCDN, each device having a plurality of
10   photosensitive pixels 102;

    b)   scanner apparatus 104 for relative movement of
        the module 110 with respect to a molecular
        sample 106 in a manner for efficient imaging;

    c)   light source 108 for exciting the molecular
15   sample;

    d)   an array driver circuit 120 for driving the
        sensor array 110 which includes clocking,
        biasing, and electronic gating of the pixel
        electrodes in CCD1...CCDN;

20   e)   a receiving circuit 122 for obtaining the
        digital image from the sensor array 110 which
        includes preamplification, amplification, analog
        to digital conversion, filtering, multiplexing,
        sampling and holding functions;

25   f)   a data processor 124 for processing the imaging
        data including contrast enhancement and
        parameter estimation; and

    g)   display means 126 for displaying and storing the
        digital image.

30   Depending upon the sample, a variety of excitation
sources 108 may be employed in the instrument. An
external x-ray source used for conventional radiography
can be employed for medical x-ray imaging. Also, UV
excitation may be provided by a lamp or laser, mounted
35 above or below the sample. Finally, for radioisotope and

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chemiluminescent labeled samples, no excitation source is
required.

    The dynamic platform embodiment of FIG. 3 enables the
imaging module or sample to be moved using a scanning
5   mechanism such as a step motor mechanically coupled to
platform 130. Thus by scanning, relatively large format
imaging can be accomplished by a smaller sensor module as
illustrated therein. A plurality of imaging devices 110
can be arranged in a module of columns to minimize
10 discontinuity. Also, the scanning can be accomplished
with intentional overlapping to provide continuous high
resolution imaging across the entire large format sample
area.

    An additional excitation method is illustrated in
15 FIG. 4 where a laser 200 is projected through a
cylindrical lens system 202 to form a beam 204 of
excitation on the sample 206 at the Brewster angle to
minimize reflective losses. Reflection 208 from the
concentrated line excitation is then imaged by the nearby
20 sensor module 210 as illustrated.

    Because physical contact may take place between the
imaging array and the substrate sample to be analyzed,
means must be used to protect the bond wires which make
contact to the imaging device to connect the pixel
25 electrodes to the driver circuits and image receiver
circuits, e.g. 120, 122 FIG. 3. These are removed from
the immediate location of the imaging array if a device
with a frame store array is used; such as described in
the Burke et al., 1991 reference. Imaging of substrates
30 of the same size as, or smaller than, the imaging array
can be accomplished with standard wire bonding and some
means to prevent the substrate from touching the device in
the neighborhood of the wire bonds. However, if the size
restriction of the substrate is removed and substrates
35 which are larger than the size of the 2xN imaging array

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are allowed, then special means of attaching and
protecting the wire bonds must be used.

     FIG. 5 shows a conventional wire bond 600 which loops
above the surface of the device to bond to a contact so
5   that contact of the wire bond with the edge 604 of the
device 602, and subsequent possible shorting, may be
avoided. FIG. 6 shows how the large sample substrate 700
must be flexed in order to contact the imaging array so
that good resolution is assured, but also clear the epoxy
10   encapsulated bond wire 600' which projects typically 15
mils above the device surface. FIG. 7 shows a new method
of making bonds in which the bond wires 600'' are brought
out approximately parallel to the surface of the device
602'. In order for there to be minimal chance for
15   shorting of the bond wire to the edge of the device 602'',
a bead 701 of epoxy is first run along the edge of the
device and is allowed to harden before attaching the
wires. Also, the land 702 on the circuit board to which
the device is bonded is positioned a distance D (the
20   proper distance) from the edge of the device so that the
wire 600'' lies nearly flat on the device surface,
protruding less than 5 mils above it, and also has a loop
sufficient to relieve stresses. FIG. 8 shows the epoxy
encapsulated wire 600''' with a substrate to be imaged 700
25   which is flexed to a much lesser extent than in FIG. 6.

     Alternatively, as in FIG. 9, in the scanning or
dynamic mode of operation, where the sample 800 is
considerably larger than the sensor array of the device
701, the sample or the sensor must be moved for imaging
30   the entire sample. Here the sensor 701 must be isolated
from the sample 800 in a manner to permit relative motion,
yet maintain the proximity requirements of lens-less
imaging. FIG. 9 shows a method which utilizes a
transparent isolation plate 22 in conjunction with the low
35   profile bonding technique previously illustrated in FIG.

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7. The distance D_i is kept minimal to enable lens-less
imaging with minimal distortion.

Conversely, a backside illuminated sensor approach
shown in FIG. 10 can be applied which enables the
5 electrical connections 900 from the device package 702 to
the sensor device 701 to be placed on the opposite side of
the device, well away from the sample 800'. Hence a
minimal distance D_b can be achieved between the sample and
imaging sensor device 701 to warrant lens-less imaging as
10 described. The backside illumination sensor approach also
provides more sensitivity since the quantum efficiency is
substantially improved by avoiding photo-electron
transmission through the polysilicon gates of the sensor
array in the device 701.
15 While this invention has been particularly shown and
described with reference to preferred embodiments thereof,
it will be understood by those skilled in the art that
various changes in form and detail may be made therein
without departing from the spirit and scope of the
20 invention as defined by the appended claims.

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CLAIMS

1. A method of detecting and forming images representing the particle emissions in an area of a sample comprising the steps of:

    a) forming an array of particle detectors, said array having a particle detector area of size commensurate with the area of the sample to be imaged;

    b) converting, in said detectors, said particle emissions emanating directly from said sample into photoelectrons;

    c) collecting said photoelectrons in said detectors; and

    d) forming an image of said collected electrons.

2. The method of Claim 1 in which said array is disposed in close proximity to said sample.

3. The method of Claim 2 in which no intervening lens structure is provided between the sample and the array.

4. The method of Claim 1 in which the array is formed of charge coupled devices.

5. The method of Claim 1 in which the array of particle detectors comprises flat panels within which an array of charge-coupled devices are formed and which panels are disposed in close proximity to said sample.

6. The method of Claim 1 in which the panels contact the sample.

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7. The method of Claim 1 in which the samples are
    labelled with substances from the group comprising
    radioisotope, fluorescent or chemiluminescent
    molecules.

5   8. The method of Claim 1 in which the sample is moved
        with respect to the array.

9. The method of Claim 1 wherein the particle detectors
    are taken from the group comprising CCD's, charge
    injection devices, amorphous silicon sensors, and
10 photodiodes.

10. A method of detecting and forming images representing
    the particle emissions in an area of a sample
    comprising the steps of:
    a) contacting the sample with an array of particle
15   detector devices;
    b) converting, in said detector devices, said
        particle emissions emanating directly from said
        sample into photoelectrons;
    c) collecting said photoelectrons in said device;
20   and
    d) forming an image of said particles from said
        collected photoelectrons.

11. The method of Claim 10 in which said array is formed
    of devices from the group comprising CCD's, CID's,
25 amorphous silicon sensors, and photodiodes.

12. The method of Claim 10 in which the sample and the
    array are moved with respect to one another.

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13. The method of Claim 10 in which the sample is labelled with material from the group comprising radioisotope, fluorescent or chemiluminescent molecules.

5 14. Apparatus for forming an image representing particle emissions in an area of a sample comprising a particle detector arranged proximal with the area of the sample to be imaged; each detector converting particle emissions, incident directly, without

10 passing through a lens, from adjacent sample portions, into photoelectrons and collecting said photoelectrons; and a display for forming an image of said detected particles from said collected photoelectrons.

15 15. The apparatus of Claim 14 wherein the image is a spatial image.

16. The apparatus of Claim 14 wherein the image is a temporal image.

17. The apparatus of Claim 14 wherein the particle
20 detector is a solid state charge-coupled device.

18. The apparatus of Claim 14 including a scanner to move the detector array or sample relative to one another.

19. Apparatus for forming an image representing particle emissions in an area of a sample comprising an
25 excitation source for exciting the sample into emitting particles, a particle detector arranged within an area of size commensurate with the area of the sample to be imaged; each detector converting particle emissions, incident directly from adjacent

-30-

sample portions, into photoelectrons and collecting
said photoelectrons; and a display for forming an
image of said collected photoelectrons.

20.   The apparatus of Claim 19 wherein the sample is in
       contact with the detector.

21.   The apparatus of Claim 19, wherein the excitation
       source is a UV light source, the detector is a CCD
       with a polysilicon gates and an optical filter.

22.   The apparatus of Claim 19 wherein the particle
       detector is a solid state charge-coupled device.

23.   The apparatus of Claim 19 including a scanner to move
       the detector array or the sample relative to one
       another.

24.   Apparatus for detection and quantification of
       particles emitted from a sample to produce a digital
       representation of the detected particles comprising
       an array of detectors for converting emissions from
       said sample to electrical signals over a wavelength
       range from about 1 to 10,000Å, and a dynamic range of
       at least 5 orders of magnitude and wherein said array
       is disposed proximal to said sample in the path of
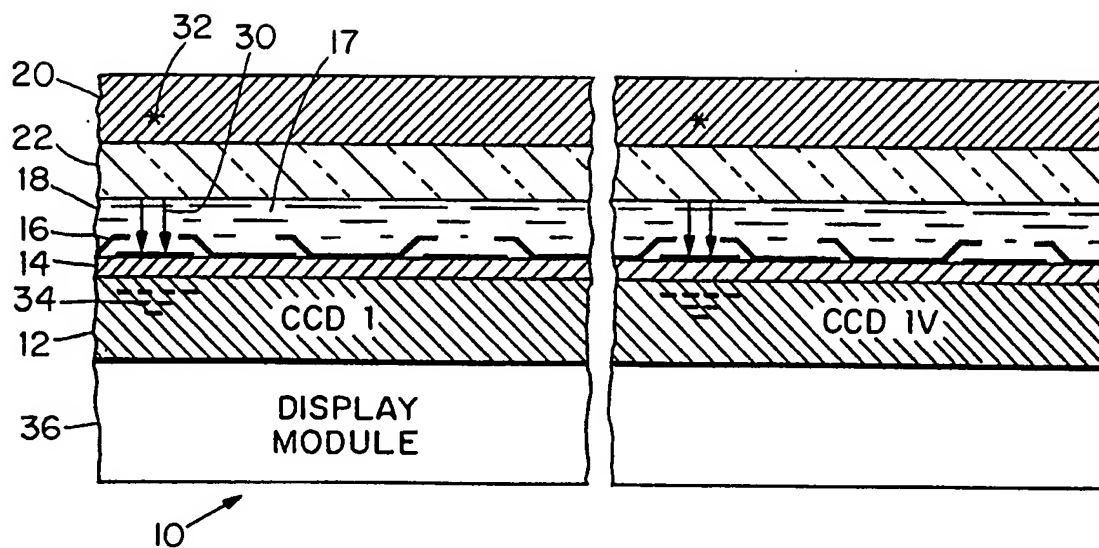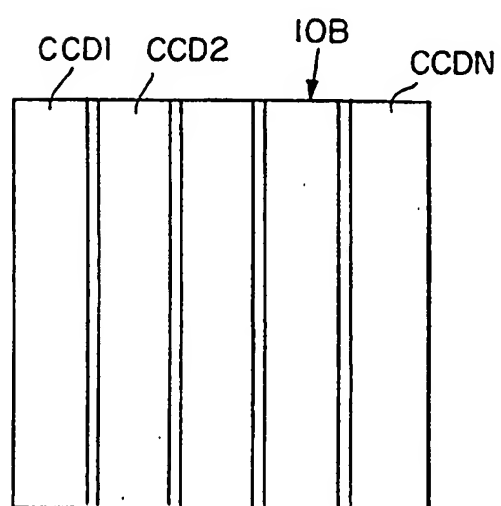   the emissions without any intervening lenses.

FIG. IA
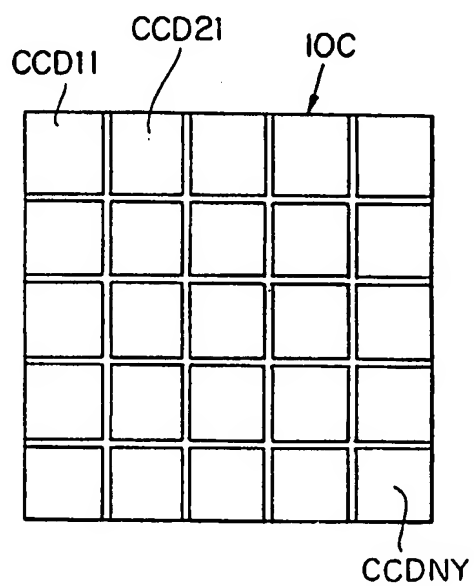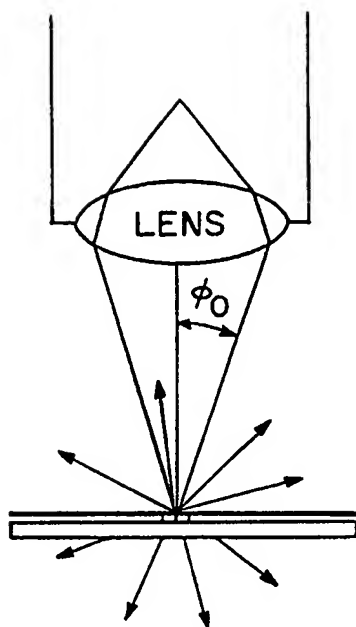

FIG. IB

FIG. IC

FIG. 2A

PRIOR ART

FIG. 2B

SAMPLE 206

200

LASER

202

204

208

LARGE FORMAT CCD MODULE 210

CCD ELECTRONICS

FIG. 4

FIG. 3

4 / 5

600

606

604   ~15mil

602

FIG. 5
PRIOR ART

EMXM

700

600′

FRAME   IMAGE
SYNCH   REGION

DEVICE 1   DEVICE 2

FIG. 6

600″

700   <4.5mil

D

FIG. 7

EPOXY   700

600‴

IMAGING
REGION

DEVICE 1   DEVICE 2
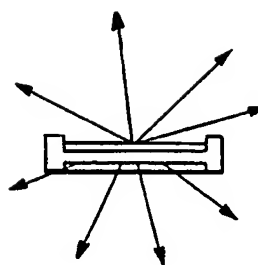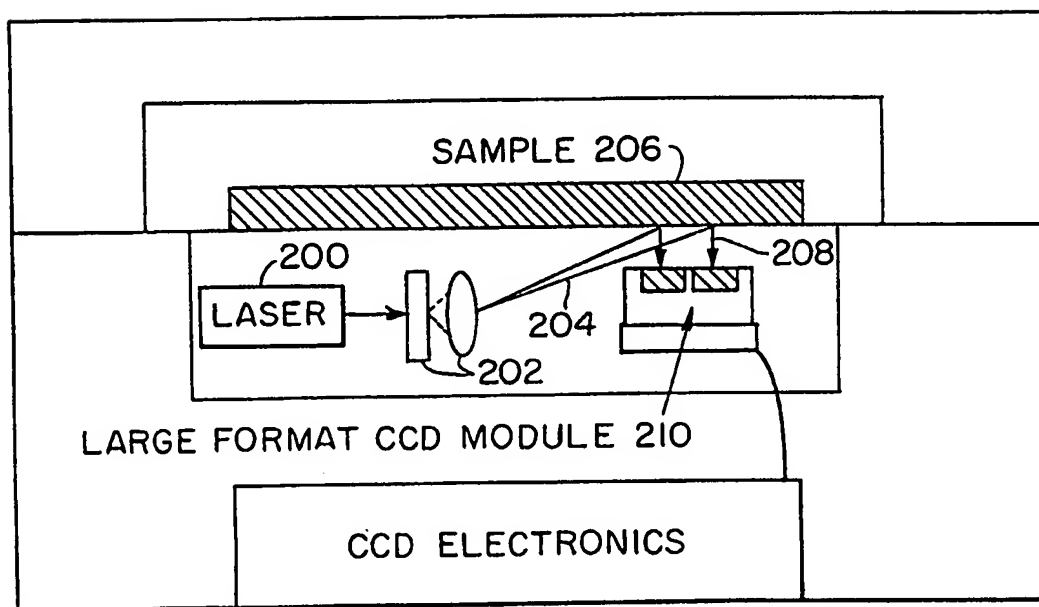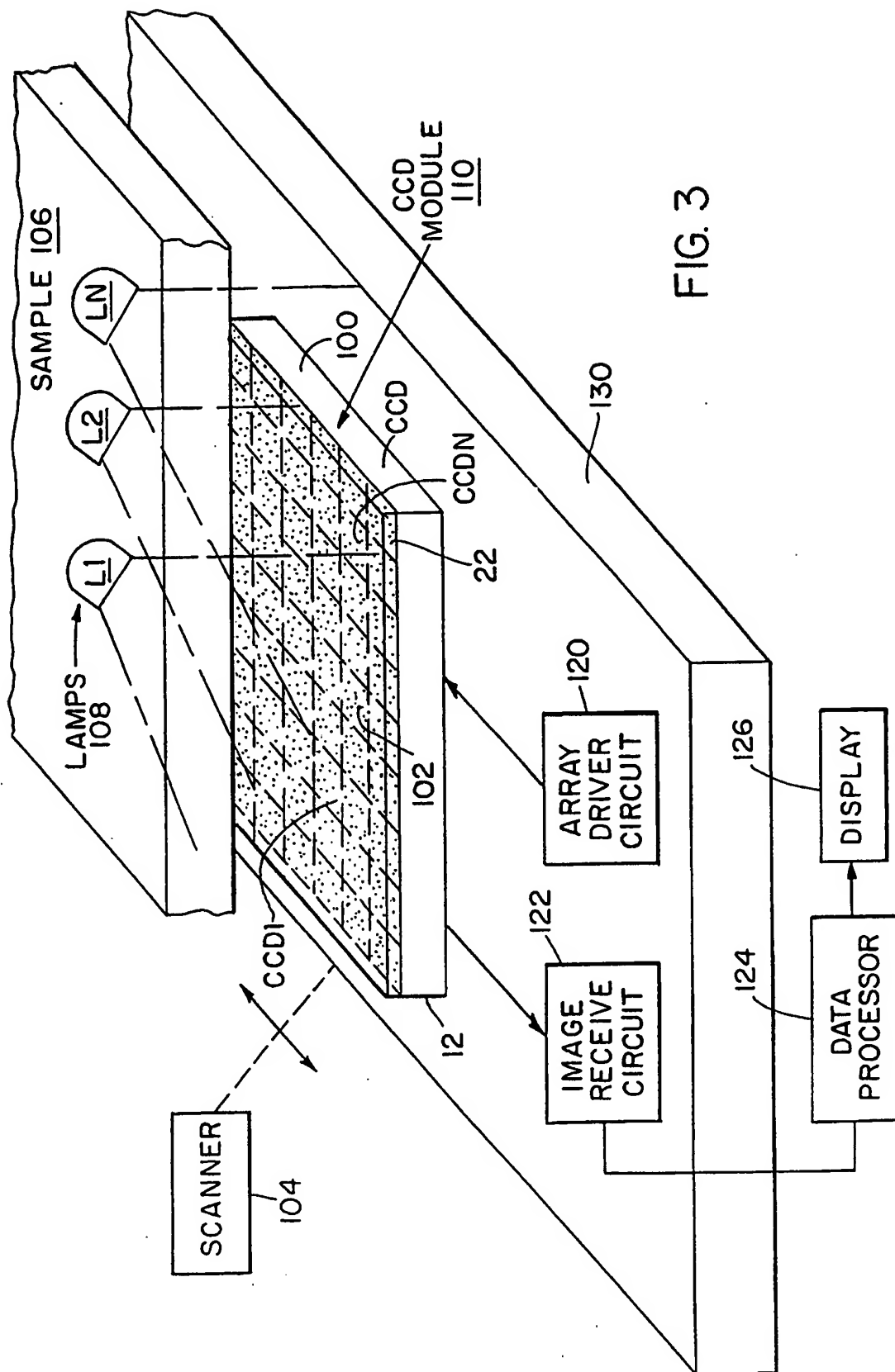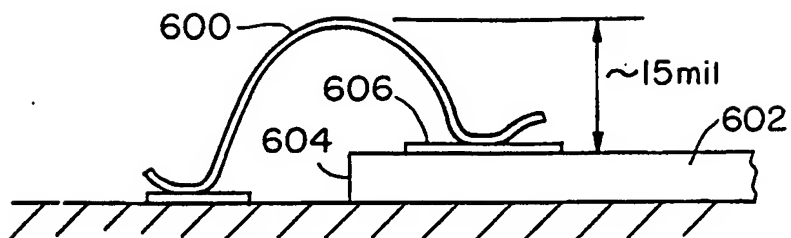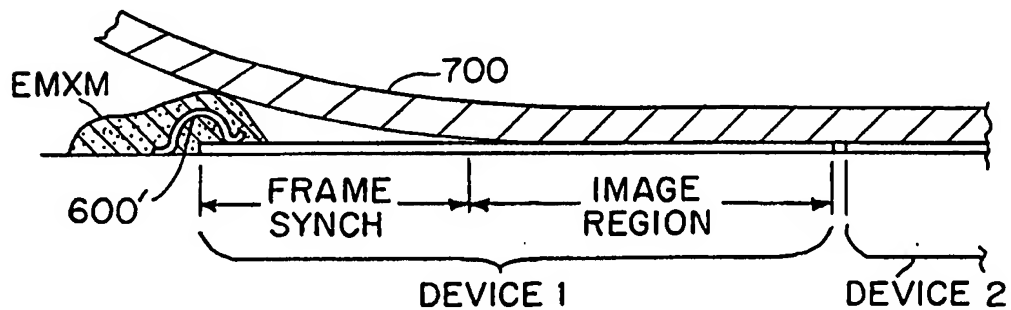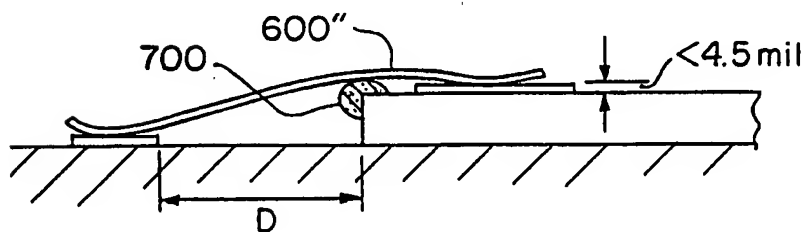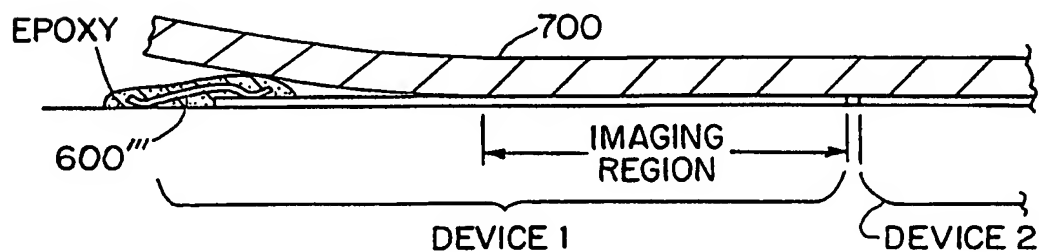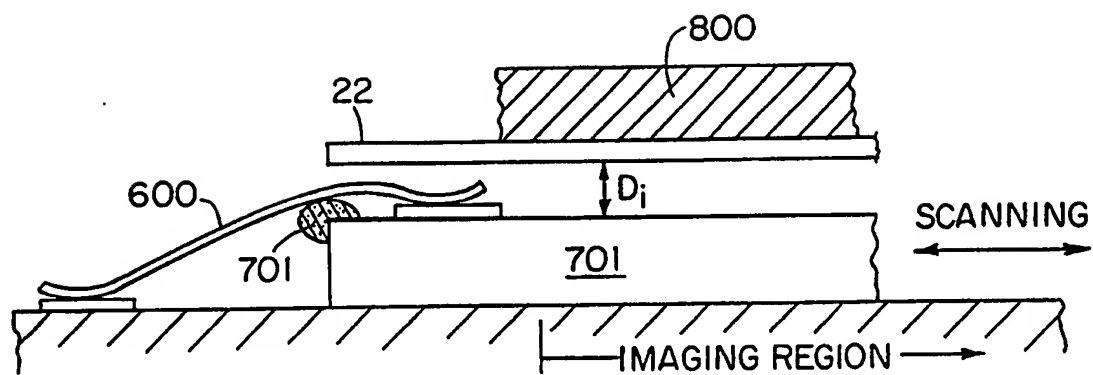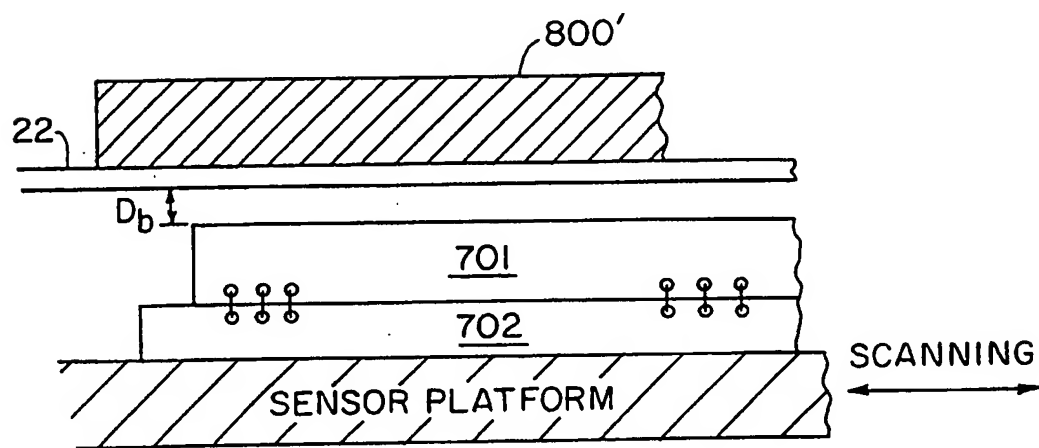
FIG. 8

FIG. 9

FIG. 10

INTERNATIONAL SEARCH REPORT

Interna l Application No
PCT/US 95/01725

A. CLASSIFICATION OF SUBJECT MATTER
G 01 T 1/29,G 01 T 1/164

According to International Patent Classification (IPC) or to both national classification and IPC 6

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A 61 B,G 01 T,G 03 G,H 04 N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ELECTROPHORESIS, vol. 9, issued 1988, JACKSON P. et al. "Rapid imaging, using a cooled CCD, of fluorescent two-dimensional polyacrylamide gels produced by labelling proteins in the first--dimensional isoelectric focusing gel with the fluorophore 2-methoxy-2,4-diphenyl--3(2H)furanone" pages 330-339, the whole document, especially 2.9 (cited in the application).	1,4,8, 9,19, 23
A	--	20,21, 22

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 June 1995	26. 07. 95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	WERNER e.h.

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ANALYTICAL CHEMISTRY, vol. 63, no. 1, issued January 1991, SWEEDLER J.V. et al. "Fluorescence Detection in Capillary Zone Electro- phoresis Using a CCD with Time-Delayed Integration" pages 496-502, the whole document, especially fig. 1-4 (cited in the application). --	1,4,7, 8,9, 19
X	US, A, 4 882 494 (ROGERS et al.) 21 November 1989 (21.11.89), the whole document, especially fig. 1,2. --	1-3,8, 14-16, 18
X	ANALYTICAL CHEMISTY, vol. 63, no. 7, issued April 1991, CHAN K.C. et al. "On-Line Detection of DNA in Gel Electrophoresis by Ultra- violet Absorption Utilizing a CCD Imaging System" pages 746-750, the whole document, especially page 747, column 2, lines 35-62.	19
A	--	20-23
X	EP, A, 0 583 118 (SUMMIT WORLD TRADE CORPORATION) 16 February 1994 (16.02.94), the whole document, especially claims 1-11; fig. 1-5. --	1-3
X	EP, A, 0 421 869 (COMMISSARIAT A L'ENERGIE ATOMIQUE) 10 April 1991 (10.04.91), the whole document, especially abstract; column 2, lines 36,37.	1
A	----	2,24

ANHANG

zum internationalen Recherchen-
bericht über die internationale
Patentanmeldung Nr.

ANNEX

to the International Search
Report to the International Patent
Application No.

ANNEXE

au rapport de recherche inter-
national relatif à la demande de brevet
international n°

PCT/US 95/01725 SAE 105734

In diesem Anhang sind die Mitglieder der Patentfamilien der im obenge- nannten internationalen Recherchenbericht angeführten Patentdokumente angegeben. Diese Angaben dienen nur zur Unter- richtung und erfolgen ohne Gewähr.

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned inter- national search report. The Office is in no way liable for these particulars which are given merely for the purpose of information.

La présente annexe indique les membres de la famille de brevets relatifs aux documents de brevets cités dans le rapport de recherche inter- national visée ci-dessus. Les reseigne- ments fournis sont donnés à titre indica- tif et n'engagent pas la responsibilité de l'Office.

Im Recherchenbericht angeführtes Patentdokument Patent document cited in search report Document de brevet cité dans le rapport de recherche		Datum der Veröffentlichung Publication date Date de publication	Mitglied(er) der Patentfamilie Patent family member(s) Membre(s) de la famille de brevets		Datum der Veröffentlichung Publication date Date de publication
US A	4882494	21-11-89	AU A1	17939/88	22-09-89
			EP A1	441772	21-08-91
			IL A0	88922	15-08-89
			WO A1	8908268	08-09-89
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			FR A1	2652655	05-04-91
			JP A2	3137589	12-06-91